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# Effects of Environmental Contaminants on Cell Mediated Immunity

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16. ABSTRACT

The effect of lead and cadmium on cell-mediated immunity was studied in peritoneal macrophages, B-, and T-lymphocytes of mice. Lead and cadmium were administered in drinking water for 10 weeks in short-term experiments and up to 18 months to deal with immune responses in aged mice.

Lead and cadmium both tended to stimulate phagocytosis in peritoneal macrophages. Consequently, depressed humoral immune response could not be explained on the basis of an effect on the macrophage. The splenic B-lymphocyte response was depressed by both lead and cadmium treatment. The direct effect of these metals on B cells could account at least in part, for the suppression of the humoral immune response reported in previous studies.

In long-term studies in aged mice low doses of lead (13 mg/l) tended to stimulate certain immune responses. Results obtained with higher doses (up to 1300 mg/l) were complicated by a natural immunosuppression in aged mice. As a consequence, no significant alterations were observed with high doses and the impact of Pb on the immune system in the long term cannot be predicted on the basis of this limited experimentation.

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EPA-600/1-78-071
December 1978

# EFFECTS OF ENVIRONMENTAL CONTAMINANTS ON CELL MEDIATED IMMUNITY

by

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# FOREWORD

Man and his environment must be protected from the adverse effects of pesticides, radiation, noise and other forms of pollution, and the unwise management of solid waste. Efforts to protect the environment require a focus that recognizes the interplay between the components of our physical environment--air, water, and land. In Cincinnati, the Environmental Research Center possesses this multidisciplinary focus through programs engaged in

- studies on the effects of environmental contaminants on man and the biosphere, and

- a search for ways to prevent contamination and to recycle valuable resources.

The Health Effects Research Laboratory conducts studies to identify environmental contaminants singly or in combination, discern their relationships, and to detect, define, and quantify their health and economic effects utilizing appropriate clinical, epidemiological, toxicological, and socioeconomic assessment methodologies.

The immune system is one of the body's primary defense systems. If a chemical interferes with the function of this system, an increased frequency of infectious disease may be expected in a population exposed to such chemicals. Newer scientific information also suggests that the immune system plays an important role in preventing tumor growth.

Previous work has shown that both lead and cadmium acted synergistically with certain infectious agents. The present work has established which components of the immune system are compromised by exposure to these metals and suggests an approach that may be useful for detecting similar effects by other environmental chemicals.

R.J. Garner
Director
Health Effects Research Laboratory

## ABSTRACT

The effect of lead and cadmium on mouse peritoneal macrophages was investigated. Lead and cadmium given orally to mice for 10 weeks stimulated phagocytosis and increased acid phosphatase levels in peritoneal macrophages. It was concluded that these two environmental contaminants activated macrophages and, therefore, the macrophage does not contribute to the immunosuppressive activity produced by lead and cadmium. The effect of lead and cadmium on macrophage related functions is discussed.

The immune response in aged mice was studied. Mice were exposed to 0, 13, or 1,300 ppm lead in drinking water for 18 months. The immunological assays performed were mitogen (lippolysaccharide E. coli, concanavalin A, and phytohemagglutinin-P) stimulation of lymphocytes; erythrocyte-antibody (EA), erythrocyte-antibody-complement (EAC), phagocytosis of macrophages; and EAC of splenic lymphocytes. As measured by the majority of these assays, the low dosage (13 ppm) of lead tended to stimulate certain immune responses (lymphocyte mitosis, EA, and EAC) while the high dosage (1,300 ppm) did not provoke any appreciable alteration. The results were interpreted by comparing data on aged mice with data on young adult mice. It was apparent from this comparison that the aged mice are naturally immunosuppressed. Therefore, the results obtained from lead-exposed aged mice were unpredictable.

B-lymphocyte response after exposure to lead and cadmium was studied. CBA/J mice were exposed to lead acetate or cadmium chloride in the drinking water for 10 weeks. The ability of bone marrow-derived lymphocytes (B cells) to form rosettes was measured using an erythrocyte-antibody-complement (EAC) assay. Fewer EAC rosettes were formed by splenic B lymphocytes from mice exposed to lead and cadmium than by B cells from control animals. The direct effect of these compounds on B cells could account in part for suppression of the humoral immune response reported in previous studies.

CBA/J mice were exposed to lead and cadmium, and the mitogen stimulation of lymphocytes was studied. Mice were given lead or cadmium in their drinking water for 10 weeks. In addition, some mice were injected with BCG. Con A, LPS, or PPD was cultured with the splenic lymphocytes. The effect of PB, Cd, and BCG on mitogen stimulation is discussed.



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CONTENTS

Foreword . . . . . iii

Abstract . . . . . iv

Tables . . . . . vi

1. Introduction . . . . . 1

2. Conclusions . . . . . 2

3. Recommendations . . . . . 5

4. Materials and Methods . . . . . 6

5. Results . . . . . 10

References . . . . . 23

Bibliography . . . . . 25

TABLES


| Number | | Page |
|---|---|---|
| 1 | Percent EAC Rosettes Formed by Spleen Cells from Mice Exposed to Lead or Cadmium for 10 Weeks | 13 |
| 2 | Percent Phagocytosis by Peritoneal Macrophages from Mice | 13 |
| 3 | Percent Viability of Peritoneal Macrophages from Mice | 14 |
| 4 | Mean Concentration of Acid Phosphatase in Peritoneal Macrophages from Mice | 14 |
| 5 | Comparison of Immunological Assay Values of Control Young Adult Mice to Aged Mice | 15 |
| 6 | Mean Body Weight of Mice | 15 |
| 7 | Mean Renal Concentrations of Lead and Cadmium | 16 |
| 8 | Lymphocyte Proliferation Response of Mice Exposed to Lead and Cadmium, Macrophages Removed | 17 |
| 9 | Lymphocyte Proliferation Response of Mice Inoculated with BCG and Exposed to Lead and Cadmium, Macrophages Removed | 18 |
| 10 | Lymphocyte Proliferation Response of Mice Inoculated with BCG and Exposed to Lead and Cadmium, Macrophage Not Removed | 19 |
| 11 | Summary of Lymphocyte Proliferation Response of Mice Exposed to Lead and Cadmium | 20 |
| 12 | Lymphocyte Stimulation Induced by LPS, Con A, and PHA from Mice Exposed to Lead for 18 Months | 21 |
| 13 | Percentage of Phagocytosis by Peritoneal Macrophages | 21 |
| 14 | Percentage of EA and EAC Rosettes Formed by Peritoneal Macrophages and Splenic Lymphocytes from Mice | 21 |
| 15 | Kidney, Liver, and Brain Concentration of Lead from Mice | 22 |

# SECTION 1

# INTRODUCTION

Cadmium (Cook et al., 1975), lead (Cook et al., 1975, Hemphill et al., 1971), methylmercury (Koller, 1975), polychlorinated biphenyls (Friend & Trainer, 1970) and sulfur dioxide (Fairchild et al., 1972) are a few of the environmental contaminants that have been demonstrated to be synergistic to infectious agents in animals.

Prolonged exposure to certain environmental contaminants may suppress the immune system of a host (Koller, 1973; Koller and Thigpen, 1973; Koller et al., 1977; Miller and Zarkower, 1974). Exposure to lead has resulted in reduced circulating antibody titers to infectious disease (Koller, 1973), decreased antibody synthesis of splenic lymphocytes (Koller and Kovacic, 1974), and inhibited formation of erythrocyte-antibody-complement (EAC) (B cell) rosettes (Koller and Brauner, 1977).

Since the macrophage has an important role as an accessory cell by co-operating with T cells in aiding the response of B cells to antigens, the effect of lead and cadmium on peritoneal exudate cells of young adult mice was investigated. The cells were examined for viability, phagocytic properties and acid phosphatase content. In addition, the response of B lymphocytes, T lymphocytes, and macrophages in aged mice chronically exposed to lead was investigated.

Subpopulations of lymphoid cells have been differentiated on the basis of characteristic cell surface antigens and receptors (Gmelig-Meyling et al., 1976; Gormus and Shands, 1975). Splenic B cells were identified by detecting their surface receptors for the activated third component of complement (C'3 receptor) (Nussenzweig, 1974). The procedure used to detect B cells involved the binding of sheep erythrocytes sensitized to antibody and complement (EAC complexes) to the C'3 receptor on B cells to form rosettes (Bianco et al., 1970). In this study, we were concerned with the effect of lead and cadmium on the B cell population in murine spleens.

Mitogens can induce blast transformation in normal lymphocytes. Concanavalin A (Con A) and phytohemaglutinin (PHA) activate thymus derived (T) lymphocytes while lipopolysaccharide E. coli (LPS) stimulates bone marrow derived (B) lymphocytes. Interference of mitogen proliferation induced by Con A and PHA suggests alteration of cell mediated immune responses while LPS indicates humoral involvement. The purpose of this study was to determine if lymphocytes collected from CBA/J mice after exposure to lead or cadmium would respond abnormally to mitogen stimulation.

## SECTION 2

## CONCLUSIONS

The support from this grant enabled us to examine the effects that lead and cadmium have on the immune system of mice. Several important features were established which revealed that lead and cadmium are generally detrimental to the immune response in animals.

First, in previous studies, we demonstrated that both lead and cadmium inhibited antibody production and thus, diminished circulating antibody titers to antigens. The lowest dosage examined (lead - 13 ppm, cadmium - 3 ppm) resulted in significant suppression.

We next wanted to determine if lead and cadmium directly affected the B lymphocyte which produces antibody. Swiss Webster mice were exposed to lead or cadmium in the drinking water for 10 weeks. The ability of bone marrow-derived lymphocytes (B cells) to form rosettes was measured using an erythrocyte-antibody-complement (EAC) assay. Fewer EAC rosettes were formed by splenic B lymphocytes from mice exposed to lead (130 or 1,300 ppm) and cadmium (30 ppm) than B cells from control animals (Table 1). The direct effect of these compounds on B cells could account in part for suppression of the humoral immune response reported in previous studies.

Macrophages indirectly influence the humoral immune system and were investigated. Lead and cadmium given orally to mice for 10 weeks stimulated phagocytosis (Table 2) and increased acid phosphatase levels (Table 4) in peritoneal macrophages. Since lead and cadmium primarily suppress the secondary immune response, the moderately reduced primary response may be due to direct action of these compounds on B cells. The increased ability of macrophages to phagocytize and contribute to immunogenicity of the antigen may not be great enough to compensate for this inhibition. Also, other factors such as opsonins are involved in *in vivo* studies. Lead and cadmium may inhibit opsonins or other soluble factors (alpha-2-globulin, etc.) that could perhaps oppose the stimulatory effect observed in the *in vitro* experiments. Finally, the results from the B cell and macrophage studies suggest that lead and cadmium may impair the complement receptor but not the Fc receptor of lymphocytes.

Concurrent with the above investigations was a study to determine the immune response in aged mice exposed to lead. Mice were exposed to 0, 13, or 1,300 ppm lead in drinking water for 18 months. The immunological assays performed were mitogen (lipopolysaccharide *E. coli*, concanavalin A, and phytohemagglutinin-P) stimulation of lymphocytes; erythrocyte-antibody (EA), erythrocyte-antibody-complement (EAC), and phagocytosis of macrophages; an

EAC of splenic lymphocytes. As measured by the majority of these assays, the low dosage (13 ppm) of lead tended to stimulate certain immune responses (lymphocyte mitosis, EA and EAC) while the high dosage (1,300 ppm) did not provoke any appreciable alteration. The results were interpreted by comparing data on aged mice with data on young adult mice (Table 5). It was apparent from this comparison that aged mice were naturally immunosuppressed. Therefore, the results obtained from lead-exposed aged mice were unpredictable.

Mitogens can induce blast transformation in normal lymphocytes. Concanavalin A (Con A) and phytohemagglutinin (PHA) activate thymus derived (T) lymphocytes while lipopolysaccharide E. coli (LPS) stimulates bone marrow derived (B) lymphocytes. Interference of mitogen proliferation induced by Con A and PHA suggests alteration of cell mediated immune responses while LPS indicates humoral involvement. Few studies concerning lymphocyte blastogenesis after exposure of a host to environmental pollutants have been conducted. The purpose of the following study was to determine if lymphocytes collected from CBA mice exposed to lead or cadmium would respond abnormally to mitogen stimulation.

Mice were exposed to 3, 30, or 300 ppm cadmium as cadmium chloride or 13, 130, 1,300 ppm lead as lead acetate orally in deionized water for 70 days. The controls were given deionized water.

Lead and cadmium had little effect on lymphocyte proliferation by Con A. Counts per minute (CPM) and relative proliferation indices (RPI) were similar for lead, cadmium and control animals from non-BCG treated mice (Table 8) and from BCG treated mice without removing macrophages (Table 10). However, when lymphocytes from BCG treated animals were used after removal of macrophages, 13 ppm lead and 30 ppm cadmium resulted in a slight increase in CPM without a noticable change in RPI (Table 9). Conversely, the low cadmium dose (3 ppm) impaired lymphocyte stimulation by Con A as seen by a decrease in CPM and RPI.

Lead reduced lymphocyte CPM and RPI by LPS from non-BCG and BCG treated mice at all levels except 13 ppm in BCG mice (Tables 8 and 9). However, when lymphocytes from BCG treated mice with macrophages were stimulated by LPS, 1,300 ppm lead enhanced CPM and RPI (Table 10). Cadmium, on the other hand, inhibited LPS stimulation of lymphocytes in most treatments at 3 ppm but resulted in significantly elevated CPM and RPI at 30 and 300 ppm (Tables 8, 9 and 10).

Response to PPD was similar to LPS. Lead in most cases decreased CPM and RPI and in some instances was as much as one-half that of the controls (Tables 1, 2 and 4). Cadmium at 3 ppm tended to inhibit CPM and RPI stimulation while a slight increase in CPM and RPI often occurred at 30 and 300 ppm cadmium dosages. In the BCG treated animals, the RPI were especially high (Table 9).

Table 11 is a summary of the mitogen studies and indicates changes for each compound and mitogen. The interpretation of proliferative responses to

mitogens has been difficult primarily due to assay variations since these measures are not stable. Furthermore, mitogens may dysfunction and vary between lots in absolute CPM which makes it difficult to combine data from identical experiments for purposes of longitudinal monitoring and analysis. Since assay variation and data analysis is a common problem encountered to measure lymphocyte function with mitogens or alloantigens by tritiated thymidine, alterations in CPM were considered significant when they were 10-15 percent larger or smaller than the control and RPI when they were greater than 1.15 or less than .85.

A summary of the work completed in the two year study revealed that lead (130 and 1,300 ppm) and cadmium (30 ppm) directly affect B lymphocytes by reducing numbers of rosettes in the EAC assay. The complement receptor site on the surface of the B cell was apparently altered by these two compounds. Lead and cadmium, on the other hand, stimulated phagocytosis and digestive functions of mouse peritoneal macrophages in vitro. However, macrophages release several soluble factors which are regulated by T cells. These factors which were not examined in this study often influence the immune response.

Finally, lead, even at low dosages, tended to inhibit mitosis of B lymphocytes while cadmium stimulated proliferation. Therefore, lead should inhibit antibody synthesis which has been demonstrated by other studies while cadmium may amplify the response. However, B lymphocytes are regulated by T cells in amount of antibody produced. T cell proliferation in this study was not altered by lead or cadmium but this technique does not directly measure helper or suppressor activity that is responsible for B cell performances.

An observation discovered during these studies is that strains of mice responded differently to lead in particular. CBA mice seem to be very resistant to lead since 1,300 ppm given orally for 18 months did not result in death. Also, antibody synthesis is not affected in these animals by lead as it is in Swiss Webster mice. We have examined other inbred strains of mice which also have not exhibited an appreciable affect to lead. Furthermore, inclusion bodies in the kidneys cannot be demonstrated by special strains as they are in other animals even though they appear to be present in H & E sections. We are currently investigating other inbred strains of mice to determine if they respond accordingly.

The smallest dose of lead used in these studies was 13.75 ppm lead in the drinking water, so the mice were ingesting about 0.069 mg of lead per day. As approximately 10 percent of ingested lead is absorbed, each mouse was actually receiving about 0.0069 mg of lead into its system each day. A significant decrease in antibody forming cells, particularly 7A, occurred at this dose. The adult human normally ingests 0.3 mg of lead per day and 2 mg per day can produce toxicity.

## SECTION 3

## RECOMMENDATIONS

I feel these studies have been valuable by providing data which helps to better understand the mechanism by which two environmental contaminants (lead and cadmium) affect the immune system of a host. These studies not only lead us closer to describing the complete mechanism of action but also provide dose response for each compound and assay. It is apparent that subclinical dosages of lead and cadmium alter the immune system of a host. Furthermore, these assays and techniques that were developed will greatly benefit future environmental investigations.

## SECTION 4

## MATERIAL AND METHODS

### ANIMALS AND DIET

Swiss Webster and CBA/J mice 28 days of age were given 3, 30, or 300 ppm cadmium as cadmium chloride or 13, 130, or 1,300 ppm lead as lead acetate orally in deionized water for 70 days. CBA/J mice were also given 13, 130, or 1,300 lead orally as lead acetate in deionized water for 18 months. The controls were given deionized water. Oregon State University Rodent Chow fed to all mice contained less than 1.12 ppm lead by analysis. There were 300 mice in each group. Mice were housed in polycarbonate cages (five per cage) with stainless steel lids and cedar shavings for bedding. These mice were free of apparent infections and ectoparasites during the experiments.

### PHAGOCYTOSIS

Five days before termination, mice were inoculated intraperitoneally (ip) with three ml mineral oil. Mice were killed by cervical dislocation and peritoneal exudate cells (PEC) were obtained by injecting seven ml cold Hank's balanced salt solution (HBSS, Flow Laboratories) containing heparin (10 U/ml) ip using a 20 gauge 1.5 in. disposable needle. Leaving the needle in place, the cavity was gently massaged and the fluid containing PEC was withdrawn. The collected PEC were washed three times on cold HBSS and centrifuged at 200 x g for 10 min. to remove excess oil. The washed pellet was transferred to a clean tube and contaminating red blood cells were lysed in 0.83% $NH_4CL$. The pellet was then diluted to six ml in culture medium composed of medium 199, 15% fetal calf serum (FCS), 100 U/ml penicillin, 100 ug/ml streptomycin, and N-2-hydroxyethyl-piperazine-N'-2-ethanesulfonic acid (HEPES) buffer, pH 7.2. Two-milliliter aliquots of the culture media were transferred to Leighton tubes (Bellco) containing a cover slip (Wheaton no. 1, 9x50mm). The cells were incubated for two hrs. at 37° C and washed once in warm phosphate-buffer saline (PBS) followed by the addition of two ml of 10% opsonized sheep red blood cells (SRBC). The preparation was incubated one hr. at 37° C.

The cover slips were then carefully removed, rinsed in two solutions of PBS, fixed in methanol, stained with Giemsa, mounted onto slides, and the cells counted. A macrophage was considered positive for phagocytosis if two or more SRBC were seen engulfed. A separate count was taken counting cells with three or more SRBC around the macrophage as in the form of a rosette. Two slides of macrophages were prepared from each mouse and 200 cells were counted per slide.

OPSONIN

To obtain optimal phagocytosis, a 10% solution of washed SRBC's was incubated for one hour with an equal amount of diluted opsonin (rabbit anti-SRBC, Cappell Laboratories, Inc.). Preliminary testing showed the optimal opsonin dilution to be 1:1600 hemagglutination units in microtiter plates. The opsonized SRBC's were washed three times in PBS and diluted to 10% in culture medium before addition to PEC monolayers.

ACID PHOSPHATASE

Acid phosphatase concentrations in macrophages were determined using a Coleman U.V. Digital Spectrophotometer. A 0.2 ml solution containing $1 \times 10^6$ macrophages was added to 1.0 ml of a buffered substrate (Boehringer Manneheim Corporation, Acid Phosphatase Test Kit, Catalog No. 15988) which contained 50 mM citrate buffer (pH 4.8), 550 u moles p-nitrophenylphosphate and 12.8 M sodium chloride. This solution was kept at 37° C for 30 min. at which time the reaction was terminated by adding 10 ml of 0.02 M sodium hydroxide. Color change was determined with the spectrophotometer at a wavelength of 405 nm by comparing treatment samples to a reagent blank.

SPLENIC LYMPHOCYTES

At termination, mice were etherized, their spleens sterilly removed, scissor minced, and smashed lightly through a stainless steel screen. The spleen cells were washed three times in cold HBSS at 200 x g for ten minutes. The spleen cell suspension was then layered on top of a Ficoll-Hypaque density gradient (Ficoll, 400,000 molecular weight, Sigma; Hypaque, 50% Winthrop) with a specific gravity of 1.09 and spun at 400 x g for thirty minutes. The interface band containing mononuclear cells was removed, washed three times in cold HBSS at 200 x g for ten minutes, counted, and checked for viability using the trypan dye exclusion test. The cells were then diluted to $1 \times 10^6$/ml in culture medium (medium 199, Flow; 10% Fetal Calf Serum, Flow; Penicillin, 100 U/ml, Squibb; Streptomycin, 100 ug/ml, Squibb; Gentamicin, 50 ug/ml; L-Glutamine 2 mM/ml, Microbiological Associates; 2 mM HEPES buffer, pH 7.2, Sigma; 2-Mercaptoethanol $5 \times 10^{-5}$M, Baker Co.). Additional mononuclear cells were plated at a cell concentration of $1 \times 10^7$ in 75 $cm^2$ tissue culture flask (Corning, #25110). The cells were incubated in culture medium at 37° C 5%$CO_2$ for two hours after which time the flasks were gently shaken to remove the non-adherent mononuclear cells. The non-adherent cells were spun, diluted to a concentration of $1 \times 10^6$/ml and pipetted 0.2 ml per microtiter well (Flow). Percent macrophages before and after removal by adherence was determined by using the non-specific esterase stain procedure of Koski (IN VITRO METHODS IN CELL-MEDIATED AND TUMOR IMMUNITY, ed. Bloom and David).

MITOGEN STIMULATION

Stock mitogens were prepared in aliquots with sterile HBSS and stored at -20° C until used. These solutions were then diluted in culture medium to the concentration required. Optimal concentrations of mitogens were

determined to be: lipopolysaccharide E. coli (LPS), 055:B5 (Difco), 160 ug/ml; LPS 0111:B4 (Difco), 40 ug/ml; phytohemagglutinin-P (PHA-P) (Difco), 4 ug/ml; and concanavalin A (Con A) (Sigma, IV), 2 ug/ml, and purified protein derivative (PPD) (Connought Laboratories, LTD.), 40 ug/ml. All cells, mitogen-stimulated and unstimulated, were cultured in quadruplicate for 72 hr. at 37° C in an atmosphere of 5% $CO_2$. At 48 hr. 0.5 uCi tritiated thymidine (6.7 Ci/mmol, New England Nuclear) was added to each well; 24 hr. later the cells were harvested using the Analog Digital Automatic Program Systems (Dedham, Massachusetts) automated system and collected on glass fiber filter paper strips (Reeve-Angel). Each filter disk was placed in a minivial (New England Nuclear) with three ml Aquasol (New England Nuclear) scintillation fluid and counted for 10 min. in a Packard Tri-Carb liquid scintillation counter (model 3375). The final values in counts per min. (CPM) were reported as the means of quadruplicate samples. The stimulation index (SI) was calculated by dividing the CPM of spleen cells with mitogen by that of spleen cells with culture medium only. A relative proliferative index (RPI) was calculated by dividing the net CPM of the test groups (mitogen stimulated CPM minus background CPM) by the net CPM of the control group (mitogen stimulated CPM minus background CPM). An RPI value greater than 1.15 was arbitrarily picked to represent an increase from the control, and a value of less than 0.85 to represent a decrease from the control. Values between 0.85 and 1.15 were considered to be of no change from the control.

MACROPHAGE EAC

The PEC's were pooled according to their respective groups and characterized by the EAC rosette assay. Sheep red blood cells were stored at 4° C in Alsever's solution for no longer than seven days. Before use, the cells were washed three times in PBS and resuspended to a final concentration of 5% Rabbit anti-sheep red blood serum (Cappel Laboratories, Inc.) was heat inactivated at 56° C for thrity min. and stored at -20° C. The hemmagglutination titer was 1:400. Autologous mouse complement serum was obtained from blood collected by cardiac puncture and stored at -70° C.

Sheep erythrocytes were sensitized with an equal volume of a 1:800 dilution of rabbit anti-sheep red blood cell serum for thirty min. at 37° C. The EA complex was an equal volume of a 1:10 dilution of mouse complement serum for thirty min. at 37° C. After two washes in VBS, the EAC complex was resuspended in HBSS, eight times the initial volume of SRBC's. A suspension of EA was prepared in a similar manner.

The PEC's ($1.5 \times 10^6$ in 15 ml HBSS) were incubated with 0.5 ml EAC at 37° C for five min. After centrifugation at 200 x five min., the cells were incubated in ice for one hr. without removing the supernatant (Jondal et al., 1972). After gentle resuspension, an aliquot of cells was mixed with an equal volume of crystal violet solution (1 mg/ml in minimal essential medium with 10% FCS). Two hundred cells were examined microscopically in a hemacytometer. Only stained cells with three or more bound erythrocytes were considered as EAC rosettes. The PEC's were similarly assayed with EA.

## LYMPHOCYTE EAC

Spleen lymphocyte cells derived from a Ficoll-Hypaque gradient were similarly characterized by the EAC rosette assay and the direct B-cell fluorescent antibody technique. Fluorescein-conjugated rabbit anti-mouse IgG (FITC-Rxmlg) (Cappel Laboratories, Inc.) was filtered through a 0.45 um Millipore filter stored at -70° C. An equal volume of spleen lymphocyte cells ($1.5 \times 10^6$ in 0.05 ml HBSS with 0.1% $NaN_3$) was incubated with the FITC-Rxmlg for thirty min. on ice. The cells were washed twice by centrifugation at 200 x g at 4° C for six min. on a gradient of 2.5 ml HBSS with 30% FCS and 0.1% $NaN_3$. After resuspension in 0.05 ml HBSS with 0.1% $NaN_3$, the cells were stored on ice until they were examined by fluorescent microscopy.

## BCG

Six weeks before termination 75 of the lead exposed, 75 of the cadmium exposed and 25 control mice were injected subcutaneously with BCG (Bacillus-Calmette Guerin; University of Illinois) diluted to 1 mg with equal amounts of CFA (Complete Freund's Adjuvant; Difco). Backs of mice were shaved and a total of four injection sites, 0.125 ml/site, or a total of 0.5 ml was injected. One week before termination the BCG treated mice received an additional injection of 0.25 mg BCG intraperitoneally.

## TISSUE RESIDUES

The brain, liver, and one kidney from each mouse was collected at necropsy and stored at -70° C for lead and cadmium analysis. These tissues were analyzed for content by atomic absorption spectrophotometry using a microcarbon furnace.

## PATHOLOGY

Sections of liver, kidney, brain, lungs, heart, urinary bladder, muscle, stomach, intestine, and spleen were collected at necropsy, fixed in 10% buffered formalin, and stained with Harris' hematoxylin and eosin (H&E). In addition, kidney sections were stained by the periodic acid-Schiff (PAS), Ziehl-Neelsen acid-fast, and Masson Trichrome methods.

## SECTION 5

## RESULTS

The percent of EAC rosette formation by spleen cells from mice exposed to lead and cadmium for 10 weeks was generally less than rosette formation by control animals (Table 1). The number of EAC rosettes was significantly less in animals which received 130 or 1,300 lead or 30 ppm cadmium. The impaired rosetting was not due to toxicity of lead and cadmium since the percent viability was similar for treated and control groups.

The inhibition of EAC rosettes produced by cadmium at 30 ppm but not 300 ppm demonstrates the variable response of animals to different dosages of cadmium which we so often observe in our laboratory. A similar response occurred with acid phosphatase concentrations in macrophages that were collected from cadmium-exposed mice (Koller and Roan, 1977).

Antibody responses to many antigens require cooperation between at least two types of lymphocytes for optimal expression. One cell type is thymus derived (T cells). T cells may amplify, help, or suppress B cells as well as function as cytoxic cells. T cells do not produce antibody. The other cell type is bone marrow derived (B cell) that mature independently of thymic influence. B cells differentiate into antibody-producing cells and are often influenced by T cells. A third cell type is the macrophage, which is important as an accessory cell in cooperating with T cells and aiding B cells in response to antigens.

Humoral antibody response to sheep red blood cells (SRBC) requires cooperation between B and T cells (Elliott and Haskill, 1974). Recent studies demonstrated suppressed antibody synthesis to SRBC in mice exposed to lead or cadmium (Koller and Kovacic, 1974; Koller et al., 1974). It was postulated that the T helper cell may be the primary target for these two environmental contaminants since the secondary immune response was most severely inhibited. However, in this study, we demonstrated that these two compounds also affected the B cells. B cells from animals exposed to lead and cadmium were inhibited in rosette formation. This could account for the diminished antibody response that was reported to occur during primary immunity after exposure to lead and cadmium (Koller and Kovacic, 1974; Koller et al., 1975). Recently it was reported that lead and cadmium fed to mice stimulated both ingestion and digestive functions of peritoneal macrophages (Koller and Roan, 1977). Studies of the effects of these contaminants on T cells are currently in progress.

Only B cells formed rosettes by the EAC technique. This was confirmed by examining nylon woll-separated T and B cells with fluorescein-conjugated

antiglobulin. All rosetted cells fluoresced with antiglobulin, indicating that they were B cells. T cells do not react with antiglobulin, nor do they form rosettes by the EAC technique. The complement receptor site on the surface of the B cell was apparently altered by lead and cadmium since lymphocyte viability and percentage T and B cells were similar in all groups.

Macrophages from CBA and Swiss Webster mice exposed to lead and cadmium were stimulated to phagocytize SRBC. Phagocytosis increased as the dose increased in the Swiss Webster mice (Table 2) while the greatest response in the CBA mice was at the high dose of both lead and cadmium and the low dose of lead. When macrophages were examined for SRBC rosettes, the response was similar for cells prepared from both treated and control animals.

Viability for macrophages was comparable for treated and control groups (Table 3). Cell viability was considered excellent for most groups and four of the six exposure groups had 96 to 99% viability.

Acid phosphatase levels were increased in macrophages of each cadmium exposure and the two highest lead exposures (Table 4). The greatest concentration of the enzyme occurred in macrophages from the medium dosages of both lead and cadmium.

The body weight of mice that received 300 ppm cadmium were significantly lower than those of animals in the other treated and control groups (Table 6). Renal concentrations of lead and cadmium increased significantly as the metal dosage increased (Table 7).

Lymphocytes from control and lead-exposed mice cultured in the presence of LPS 055:B5, LPS 0111:B4, Con A and PHA-P were classified according to the SI values (Table 12). The SI values for LPS 055:B5, LPS 0111:B4, and Con A were markedly elevated in mice exposed to 13 ppm lead compared to controls or to mice given 1,300 ppm lead. However, the SI of PHA-P was elevated in the mice exposed to 1,300 ppm lead compared to controls or to mice exposed to 13 ppm lead.

The ability of macrophages to phagocytize SRBC's was inhibited in mice exposed to 13 ppm lead (Table 13). However, the EA and EAC values for macrophages (Table 14) and lymphocytes were slightly elevated in the same 13 ppm lead group where as they were suppressed in the 1,300 ppm group compared to controls. Lymphocyte viability before culturing for mitogen stimulation and the EAC test was generally 85-95%.

Tissue residues of lead increased with an increase in dosage (Table 15). The kidneys contained the largest quantities of lead in both exposures; larger quantities of lead were found in the brain than in the liver in the 13 ppm group, while the converse was found in the 1,300 ppm group.

Histopathology disclosed a wide zone of necrosis at the cortical medullary junction in the kidneys of mice exposed to 1,300 ppm lead. The principle lesion was necrosis of tubular epithelial cells. Within these necrotic areas there were frequent granulomas composed of large mononuclear cells, occasional

multinucleated giant cells, and fibrous connective tissue. Many intranuclear inclusion-like bodies were identified in H&E sections, but they did not stain well by the acidfast method.

The lesions in mice exposed to 13 ppm lead were much less severe and were characterized by necrosis of the tubular epithelial cells at the cortical medullary junctions. Granulomas were infrequent in these lesions. The PAS stain demonstrated thickening of the glomerular basement membranes and Bowman's capsule in the lead-exposed animals. Masson's trichrome stain revealed a slight increase of fibrous connective tissue in the necrotic areas of the kidneys of many of the lead-exposed mice. Spontaneous tumors were found in four lead-exposed mice and in one control animal.

Lead and cadmium had little effect on lymphocyte proliferation by Con A. Counts per minute (CPM) and relative proliferation indexes (RPI) were similar for lead, cadmium and control animals when lymphocytes were examined from non-BCG treated mice (Table 8) and from BCG treated mice without removing macrophages (Table 10) in the lymphocyte suspensions. However, when lymphocytes from BCG treated animals were used after removal of macrophages, 13 ppm lead and 30 ppm cadmium resulted in a slight increase in CPM without a noticeable change in RPI (Table 9). Conversely the low cadmium dose (3 ppm) impaired lymphocyte stimulation by Con A reflected by both decreased CPM and RPI.

Lead reduced lymphocyte CPM and RPI by LPS from non-BCG and BCG treated mice at all levels except 13 ppm in BCG mice (Tables 8 and 9). However, when lymphocyte from BCG treated mice with macrophages were stimulated by LPS, 1,300 ppm lead enhanced CPM and RPI (Table 10). Cadmium, on the other hand, inhibited LPS stimulation of lymphocytes in most treatments at 3 ppm but resulted in significantly elevated CPM and RPI at 30 and 300 ppm (Tables 8, 9, and 10).

Response to PPD was similar to LPS. Lead in most cases decreased CPM and RPI and in some instances were as much as one half that of the controls (Tables 8, 9, and 10). Cadmium at 3 ppm tended to inhibit CPM and RPI of PPD stimulation while a slight increase in CPM and RPI often occurred at 30 and 300 ppm cadmium dosages. In the BCG treated animals, the RPI were especially high (Table 9).

Cell viability averaged 87 percent from non-BCG mice and slightly less (84%) for the BCG treated animals. Most of the macrophages were removed by the Ficoll-Hypaque density gradient but for the non-BCG and BCG without macrophate groups, the cells were plated in plastic flasks for two hours. Two to three percent were present after plating. However, in the 300 ppm cadmium group, eight percent macrophages were present before plating and three percent after.

TABLE 1. PERCENT EAC ROSETTES FORMED BY SPLEEN CELLS FROM MICE EXPOSED TO LEAD OR CADMIUM FOR 10 WEEKS +

| Dose (ppm) Lead | Dose (ppm) Cadmium | EAC (%) Lead | EAC (%) Cadmium |
|---|---|---|---|
| 0 | 0 | 53.5 | 45.1 |
| 13 | 3 | 46.5 | 42.9 |
| 130 | 30 | 41.5*[1] | 30.9*[2] |
| 1,300 | 300 | 41.3*[1] | 42.7 |

+ = 25 mice per group

* = Significant $P < 0.05$ (one way analysis of variance)

[1] = LSD - 9.64 [2] = LSD - 13.63

TABLE 2. PERCENT PHAGOCYTOSIS BY PERITONEAL MACROPHAGES FROM MICE EXPOSED TO LEAD OR CADMIUM FOR 10 WEEKS

| Dose (ppm) | | Phagocytosis (%) | | | |
|---|---|---|---|---|---|
| | | CBA | | SW | |
| Lead | Cadmium | Lead | Cadmium | Lead | Cadmium |
| 0 | 0 | 56 | 54 | 43 | 43 |
| 13 | 3 | 67 | 56 | 49 | 38 |
| 130 | 30 | 54 | 56 | 57* | 45 |
| 1,300 | 300 | 72* | 63* | 62** | 59* |

* = Significant at $P < 0.05$ (t test)

** = Significant at $P < 0.01$ (t test)

TABLE 3. PERCENT VIABILITY OF PERITONEAL MACROPHAGES FROM MICE EXPOSED TO LEAD OR CADMIUM FOR 10 WEEKS

| Dose (ppm) | | Macrophage viability (%)[a] | |
|---|---|---|---|
| Lead | Cadmium | Lead | Cadmium |
| 0 | 0 | 94 | 93 |
| 13 | 3 | 96 | 89 |
| 130 | 30 | 96 | 99 |
| 1,300 | 300 | 89 | 96 |

[a] = Ten mice in each exposure group; 2 samples per mouse

TABLE 4. MEAN CONCENTRATION OF ACID PHOSPHATASE IN PERITONEAL MACROPHAGES FROM MICE EXPOSED TO LEAD OR CADMIUM FOR 10 WEEKS

| Dose (ppm) | | Concentration acid phosphatase[a] | |
|---|---|---|---|
| Lead | Cadmium | Lead | Cadmium |
| 0 | 0 | 18.37 | 21.72 |
| 13 | 3 | 12.81 | 28.12 |
| 130 | 30 | 57.63** | 34.80* |
| 1,300 | 300 | 24.50 | 28.40 |

[a] = SI units

* = Significant at $P < 0.05$ (t test)

** = Significant at $P < 0.05$ (t test)

TABLE 5. COMPARISON OF IMMUNOLOGICAL ASSAY VALUES OF CONTROL YOUNG ADULT MICE TO AGED MICE[a]

| Immunological assay | Young adult mice[b] | Aged mice |
|---|---|---|
| Phagocytosis by macrophages (%) | 56 | 40 |
| EAC lymphocyte rosettes (%) | 54 | 43 |
| Lymphocyte stimulation index | | |
| LPS 055:B5 | 27.5 | 6.9 |
| LPS 0111:B4 | 17.9 | 4.6 |
| Con A | 32.6 | 9.3 |
| PHA-P | 3.2 | 1.8 |

[a]Erythrocyte-antibody-complement (EAC)
Lipopolysaccharide E. coli (LPS)
Concanavalin A (Con A)
Phytohemagglutinin-P (PHA-P)

[b]Young adult mice were 14 weeks old

TABLE 6. MEAN BODY WEIGHTS OF MICE EXPOSED TO LEAD OR CADMIUM FOR 10 WEEKS

| Dose (ppm) | | Body weight (g)[a] | |
|---|---|---|---|
| Lead | Cadmium | Lead | Cadmium |
| 0 | 0 | 26 | 27 |
| 13 | 3 | 27 | 25 |
| 130 | 30 | 27 | 26 |
| 1,300 | 300 | 24 | 18** |

[a] = Ten mice each exposure group

** = Significant at $P < 0.01$

TABLE 7. MEAN RENAL CONCENTRATIONS OF LEAD AND CADMIUM FROM MICE EXPOSED FOR 10 WEEKS

| Dose (ppm) | | Renal concentration (ppm)[a,b] | |
|---|---|---|---|
| Lead | Cadmium | Lead | Cadmium |
| 0 | 0 | 0.07 | 0.31 |
| 13 | 3 | 0.35 | 1.07 |
| 130 | 30 | 1.29 | 8.35 |
| 1,300 | 300 | 8.10 | 44.80 |

[a] Wet weight

[b] Ten mice in each exposure group

TABLE 8. LYMPHOCYTE PROLIFERATION RESPONSE OF MICE EXPOSED TO LEAD AND CADMIUM, MACROPHAGES WERE REMOVED FROM THE LYMPHOCYTE SUSPENSIONS BY PLATING

| Dosage | Con A | | | LPS | | | PPD | | |
|---|---|---|---|---|---|---|---|---|---|
| | CPM[1] (CV) | SI[2] (CV) | RPI[3] (CV) | CPM[1] (CV) | SI[2] (CV) | RPI[3] (CV) | CPM[1] (CV) | SI[2] (CV) | RPI[3] (CV) |
| Control | 108,000 (.36)[4] | 47 (.58) | | 38,000 (.49) | 15 (.27) | | 27,500 (.08) | 7 (.20) | |
| Lead | | | | | | | | | |
| 13 | 100,000 (.23) | 79 (.66) | 1.01 (.40) | 25,000 (.08) | 19 (.53) | 0.80 (.56) | 13,000 (.24) | 12 (.71) | 0.52 (.41) |
| 130 | 105,000 (.17) | 64 (.39) | 1.08 (.42) | 28,000 (.22) | 17 (.47) | 0.88 (.52) | 19,500 (.33) | 13 (.62) | 0.77 (.49) |
| 1300 | 119,000 (.25) | 52 (.35) | 1.18* (.38) | 31,000 (.65) | 11 (.23) | 0.95 (.64) | 18,000 (.86) | 6 (.13) | 0.66 (.93) |
| Cadmium | | | | | | | | | |
| 3 | 102,000 (.23) | 52 (.18) | 1.04 (.45) | 26,000 (.12) | 13 (.04) | 0.84 (.61) | *14,500 (.05) | 7 (.20) | *0.51 (.14) |
| 30 | 109,000 (.12) | 29 (.35) | 1.10 (.37) | 55,000 (.28) | 14 (.19) | 1.61 (.34) | 32,500 (.11) | 6.5 (.33) | 1.15 (.06) |
| 300 | 105,000 (.07) | 24 (.19) | 1.05 (.38) | *64,000 (.14) | 15 (.17) | 2.17 (.71) | 32,500 (.11) | .9 (.04) | 1.22 (.22) |
| | (.20) | (.39) | (.40) | (.28) | (.27) | (.56) | (.25) | (.32) | (.38) |

1 = mean counts per minute

2 = mean stimulation index

3 = mean relative proliferative index

4 = coefficient of variance

* = Significantly different from control population at $P < 0.05$

TABLE 9. LYMPHOCYTE PROLIFERATION RESPONSE OF MICE INOCULATED WITH BCG AND EXPOSED TO LEAD AND CADMIUM. MACROPHAGES WERE REMOVED FROM THE LYMPHOCYTE SUSPENSIONS BY PLATING

| Dosage (ppm) | Con A | | | LPS | | | PPD | | |
|---|---|---|---|---|---|---|---|---|---|
| | CPM[1] (CV) | SI[2] (CV) | RPI[3] (CV) | CPM[1] (CV) | SI[2] (CV) | RPI[3] (CV) | CPM[1] (CV) | SI[2] (CV) | RPI[3] (CV) |
| Control | 125,000 (.18)[4] | 43 (.53) | | 52,500 (.48) | 15 (.14) | | 9,000 (.56) | 2.5 (.40) | |
| Lead | | | | | | | | | |
| 13 | 139,000 (.18) | 38 (.33) | 1.13 (.22) | 54,000 (.36) | 14 (.12) | 1.12 (.38) | 11,000 (.92) | 2.4 (.44) | 1.09 (.52) |
| 130 | 133,000 (.26) | 52 (.27) | 1.09 (.29) | 41,000 (.26) | 16 (.21) | 0.98 (.59) | 5,000 (.74) | 1.8 (.54) | 0.54 (1.42) |
| 1300 | 126,000 (.15) | 56 (.30) | 1.03 (.17) | 37,000 (.34) | 15 (.12) | 0.77 (.33) | 4,000 (.30) | 1.8 (.12) | 0.40 (.36) |
| Cadmium | | | | | | | | | |
| 3 | 108,000 (.28) | 40 (.23) | 0.86 (.17) | 49,000 (.29) | 19 (.36) | 1.16 (.56) | 7,000 (.68) | 2.7 (.55) | 0.93 (.97) |
| 30 | 139,000 (.16) | 33 (.67) | 1.07 (.08) | 67,000 (.44) | 15 (.15) | 1.31 (.21) | 12,000 (.50) | 2.5 (.42) | 1.34 (.08) |
| 300 | 128,000 (.19) | 39 (.50) | 1.03 (.16) | 61,000 (.30) | 17 (.24) | 1.44 (.52) | 9,000 (.29) | 2.4 (.35) | 1.52 (.96) |
| | (.20) | (.40) | (.19) | (.35) | (.19) | (.43) | (.57) | (.40) | (.72) |

[1] = mean counts per minute

[2] = mean stimulation index

[3] = mean relative proliferative index

[4] = coefficient of variance

TABLE 10. LYMPHOCYTE PROLIFERATION RESPONSE OF MICE INOCULATED WITH BCG AND EXPOSED TO LEAD AND CADMIUM. MACROPHAGES WERE NOT REMOVED FROM THE LYMPHOCYTE SUSPENSION BY PLATING

| Dosage (ppm) | Con A | | | LPS | | | PPD | | |
|---|---|---|---|---|---|---|---|---|---|
| | CPM[1] (CV) | SI[2] (CV) | RPI[3] (CV) | CPM[1] (CV) | SI[2] (CV) | RPI[3] (CV) | CPM[1] (CV) | SI[2] (CV) | RPI[3] (CV) |
| Control | 169,000[4] (.07) | 40 (.29) | | 58,000 (.35) | 13 (.12) | | 11,000 (.30) | 2.6 (.07) | |
| Lead | | | | | | | | | |
| 13 | 170,000 (.11) | 41 (.07) | 1.01 (.12) | 53,000 (.28) | 13 (.16) | 0.93 (.17) | 11,000 (.49) | 2.5 (.36) | 0.85 (.40) |
| 130 | 181,000 (.16) | 47 (.12) | 1.07 (.13) | 54,000 (.04) | 15 (.25) | 1.00 (.31) | 5,500 (.63) | 2.1 (.03) | 0.68 (.47) |
| 1300 | 166,000 (.07) | 36 (.21) | 0.98 (.13) | 67,000 (.29) | 14 (.12) | 1.17 (.06) | 13,000 (.44) | 2.8 (.31) | 1.16 (.22) |
| Cadmium | | | | | | | | | |
| 3 | 172,000 (.10) | 38 (.32) | 1.02 (.11) | 53,000 (.33) | 11 (.19) | 0.90 (.15) | 10,000 (.45) | 2.1 (.24) | 0.77 (.42) |
| 30 | 178,000 (.12) | 30 (.99) | 1.03 (.06) | 76,000 (.45) | 10 (.25) | 1.29 (.45) | 12,000 (.54) | 2.3 (.27) | 1.12 (.12) |
| 300 | 177,000 (.10) | 25 (.14) | 1.03 (.10) | 78,000 (.13) | 11 (.14) | 1.42 (.33) | 17,000 (.50) | 2.3 (.50) | 1.49 (1.02) |
| | (.10) | (.31) | (.11) | (.27) | (.18) | (.25) | (.48) | (.25) | (.44) |

1 = mean counts per minute

2 = mean stimulation index

3 = mean relative proliferative index

4 = coefficient of variance

TABLE 11. SUMMARY OF LYMPHOCYTE PROLIFERATION RESPONSE OF MICE EXPOSED TO LEAD AND CADMIUM. GROUP 2 AND 3 IN ADDITION WERE INOCULATED WITH BCG WHILE GROUP 2 ALSO HAD MACROPHAGES REMOVED FROM THE LYMPHOCYTE SUSPENSION BY PLATING

| | | Concanavalin A | | | | | |
|---|---|---|---|---|---|---|---|
| | | Non-BCG | | BCG | | BCG w/Macs | |
| Lead | Cadmium | CPM | RPI | CPM | RPI | CPM | RPI |
| 13 | | - | - | - | - | - | - |
| 130 | | - | - | - | - | - | - |
| 1300 | | - | I | - | - | - | - |
| | 3 | - | - | - | - | - | - |
| | 30 | - | - | - | - | - | - |
| | 300 | - | - | - | - | - | - |
| | | Lipopolysaccharide | | | | | |
| 13 | | D | D | - | - | - | - |
| 130 | | D | - | D | - | - | - |
| 1300 | | D | - | D | D | I | I |
| | 3 | D | D | - | I | - | - |
| | 30 | I | I | I | I | I | I |
| | 300 | I | I | I | I | I | I |
| | | Purified Protein Derivative | | | | | |
| 13 | | D | D | - | - | - | D |
| 130 | | D | D | D | D | D | D |
| 1300 | | D | D | D | D | I | I |
| | 3 | D | D | D | - | - | D |
| | 30 | I | - | I | I | - | - |
| | 300 | I | I | - | I | I | I |

I = increase
D = decrease
- = no significant change

TABLE 12. LYMPHOCYTE STIMULATION INDUCED BY LPS, CON A, AND PHA FROM MICE EXPOSED TO LEAD FOR 18 MONTHS[a]

| Lead (ppm) | Stimulation index | | | |
|---|---|---|---|---|
| | LPS 055:B5 | LPS 0111:B4 | Con A | PHA-P |
| 0 | 6.9 | 4.6 | 9.3 | 1.8 |
| 13 | 11.1 | 9.0[b] | 12.2 | 2.0 |
| 1,300 | 7.9 | 5.7 | 7.1 | 2.7 |

[a] Thirty mice per group; LPS, lipopolysacchride E. coli; Con A, concanavalin A; PHA-P, phytohemagglutinin-P

[b] Significant at $P < 0.05$ (one-way analysis of variance; LSD = 4.27)

TABLE 13. PERCENTAGE OF PHAGOCYTOSIS BY PERITONEAL MACROPHAGES FROM MICE EXPOSED TO LEAD FOR 18 MONTHS

| Lead (ppm) | Phagocytosis (%) |
|---|---|
| 0 | 40 |
| 13 | 32[b] |
| 1,300 | 42 |

[a] Thirty mice per group

[b] Significant at $P < 0.05$ (one-way analysis of variance; LSD = 5.86)

TABLE 14. PERCENTAGE OF EA AND EAC ROSETTES FORMED BY PERITONEAL MACROPHAGES AND SPLENIC LYMPHOCYTES FROM MICE EXPOSED TO LEAD FOR 18 MONTHS

| Lead (ppm) | Macrophages | | Lymphocytes |
|---|---|---|---|
| | EA (%) | EAC (%) | EAC (%) |
| 0 | 53 | 57 | 44 |
| 13 | 55 | 59 | 45 |
| 1300 | 50 | 52 | 39 |

[a] EA, erythrocyte-antibody; EAC, erythrocyte-antibody-complement

TABLE 15. KIDNEY, LIVER, AND BRAIN CONCENTRATIONS OF LEAD FROM MICE EXPOSED FOR 18 MONTHS

| Lead (ppm) | Mean lead (ppm) | | |
|---|---|---|---|
| | Brain | Liver | Kidney |
| 0 | 0.10 | 0.10 | 0.14 |
| 13 | 0.51 | 0.25 | 0.97 |
| 1300 | 5.94 | 7.32 | 17.79 |

[a] Wet weight

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15. SUPPLEMENTARY NOTES

16. ABSTRACT

The effect of lead and cadmium on cell-mediated immunity was studied in peritoneal macrophages, B-, and T-lymphocytes of mice. Lead and cadmium were administered in drinking water for 10 weeks in short-term experiments and up to 18 months to deal with immune responses in aged mice.

Lead and cadmium both tended to stimulate phagocytosis in peritoneal macrophages. Consequently, depressed humoral immune response could not be explained on the basis of an effect on the macrophage. The splenic B-lymphocyte response was depressed by both lead and cadmium treatment. The direct effect of these metals on B cells could account at least in part, for the suppression of the humoral immune response reported in previous studies.

In long-term studies in aged mice low doses of lead (13 mg/l) tended to stimulate certain immune responses. Results obtained with higher doses (up to 1300 mg/l) were complicated by a natural immunosuppression in aged mice. As a consequence, no significant alterations were observed with high doses and the impact of Pb on the immune system in the long term cannot be predicted on the basis of this limited experimentation.

17. KEY WORDS AND DOCUMENT ANALYSIS

| a. DESCRIPTORS | b. IDENTIFIERS/OPEN ENDED TERMS | c. COSATI Field/Group |
|---|---|---|
| Lead (metal)<br>Cadmium<br>Lymphocytes<br>Toxicity<br>Mice | Immune response<br>Peritoneal macrophage | 13B |

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